REMARKS

Claims 1-127 are pending in the application. Claims 1-11, 14-40 and 53-89 have been rejected. Claims 1, 4, 5, 6, 8, 14 and 53 have been amended. Support for these amendments is found throughout the specification. Examples of support are found below in the arguments. No new matter has been added by virtue of these amendments and their entry is respectfully requested.

A Request for Continued Examination and appropriate fee is filed herewith, due to the finality of the Office Action.

Claim Rejections Under 35 U.S.C. §112

Claims 4-6 and 8 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants have amended the claims as per the Examiner's recommendations.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claims 1-11, 14-40, and 53-89 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

Applicants respectfully traverse.

However, to expedite prosecution, applicants have amended independent claims 1, 14 and 53 to recite: "a first carboxy-terminal portion of a split intein (C-intein), a second amino-terminal portion of a split intein (N-intein), and a target peptide flanked on one end

with the carboxy-terminal portion of a split intein (C-intein) and on its other end with the amino-terminal portion of a split intein (N-intein)..." Claims 2-11, depend from claim 1; claims 14-40 depend on claim 14 and claims 54-89 depend on claim 53. Support for these amendments is found throughout the specification. See, for example, page 18, lines 5-23 through to page 19, lines 1-12; see, also for example: on page 5, lines 3-12, Applicants describe the method for producing and screening of cyclic peptide libraries *in vivo*:

> A general method for the *in vivo* production and screening of cyclic peptide libraries has been discovered. In this method, a nucleic acid molecule is constructed such that a nucleotide sequence encoding the peptide to be cyclized is flanked on one end with a nucleotide sequence encoding the **carboxy-terminal portion** of a split (or trans) intein (C-intein or I_C) and on its other end with a nucleotide sequence encoding the **amino-terminal portion** of a split intein (N-intein or I_N). Expression of the construct within a host system such as a bacterium or eukaryotic cell results in the production of a fusion protein. The two split intein components (i.e., I_C and I_N) of the fusion protein then assemble to form an active enzyme that splices the amino and carboxy termini together to generate a backbone cyclic peptide. (Emphasis added).

Applicants respectfully disagree with the Examiner regarding requiring a written description of members of each genus. Applicants provide sufficient written description as to selection of inteins, in view of the amended claims. For example, a first portion and a second portion is an N-terminal portion of a split intein and a carboxy-terminal portion of a split intein wherein each portion alone has no activity and requires the assembly of these portions to form an active enzyme. The number of amino acids that comprise each portion is not important, but the fact that assembly of each portion must form an **active** enzyme. Applicants further teach examples of split inteins. See, for example, page 6, lines 16-19:

> Both the **first portion** of a split intein and the **second portion** of a split intein can be derived from a naturally-occurring split intein such as Ssp DnaE. In other variations, one or both of split intein portions can be derived from non-naturally occurring

split inteins such as those derived from RecA, DnaB, Psp Pol-I, and Pfu inteins. (Emphasis added).

Applicants teach that the N- and C-terminal inteins are proteins that actually associate to form a complex that initiates and drives the cyclization reaction. (See for example, page 18, lines 5-23 through to page 2, lines 1-12 and figures 1 and 2). Within this complex the cyclization reaction occurs with the concomitant loss of the N- and C-terminal inteins. Applicants also teach that the portions of split inteins can be naturally occurring or artificially produced. See, for example, page 20, lines 11-24 through to page 21, lines 1-13:

> Nucleotide sequences that encode the first portion of a split intein and the second portion of a split intein of the nucleic acid molecules within the invention can be derived from **known inteins.** A fairly comprehensive and descriptive list of such inteins is published by New England Biolabs at http//www.neb.conljinteins/ifltreghtml **Any of these known inteins can be used as long as they are compatible with invention**.
>
> Nucleotide sequences that encode either naturally-occurring or artificially-produced split inteins can be used to generate the intein portions of nucleic acid molecules within the invention. Naturally-occurring split inteins are expressed in nature as two separate components that bind one another to form one active splicing agent. The nucleic acid molecules encoding these naturally-occurring components can thus be used in the invention. One example of a naturally-occurring split intein that may be used is Ssp DnaE (Wu et al, Proc. Natl. Acad. Sci. USA 95:9226,1998).
>
> Inteins that are not split in their natural state (i.e., those that exist as one continuous chain of amino acids) can be artificially split using known techniques. For example, two or more nucleic acid molecules encoding different portions of such inteins can be made so that their expression yields two or more artificially split intein components. See, e.g., Evans *et al, J. Biol. Chem.* 274:18359, 1999; Mills *et al, Proc. Natl. Acad. Sci.* USA 95:3543, 1998. The nucleic acids that encode such non-naturally occurring intein **components (portions)** can be used in the invention. Those nucleic acid molecules that encode non-naturally occurring split intein portions which efficiently interact on the same precursor polypeptide to yield

> cyclic peptides or splicing intermediates are preferred. Examples of non-naturally occurring split inteins from which such nucleic acid molecules can be derived include Psp Pol- I (Southworth, M.W., et al, *The EMBO J.* 17:918, 1998), Mycobacterium tuberculosis RecA intein, (Lew, B.M., *et al, J. Biol. Chem.* 273:15887, 1998; Shingledecker, K., *et al, Gene* 207:187, 1998; Mills, K.V., *et al, Proc. Natl. Acad. Sci.* USA 95:3543, 1998), Ssp DnaB/Mxe GyrA (Evans, T.C. *et al, J. Biol. Chem.* 274:18359, 1999), and Pfu (Otomo *et al, Biochemistry* 38:16040, 1999; Yamazaki *et al, J. Am. Chem. Soc.* 120:5591, 1998). (Emphasis added).

Applicants submit, that known inteins can be used based on the instant teachings. Examples are taught within the specification and in the originally filed claims. Applicants respectfully disagree with the Examiner's assertions that "the disclosure of these polynucleotides is insufficient to be representative of the attributes and features of all species encompassed by the claims." The sequences of these inteins are known and the common structural feature which is inherent of each intein is the ability to be cleaved and reassemble. Applicants further describe, in detail, the invention and that inteins (RecA, DnaB, Psp, Pol-I or Pfu inteins) as claimed, are suitable in the methods of the invention. These inteins can be used to produce multiple split inteins. Indeed, knowledge of inteins in the art, is such that the mere recitation of the word "intein" immediately conjures a genus of functionally equivalent protein sequences in the mind of the person of skill in the art which, when provided with the teachings of the present disclosure, readily allows the artisan to make and use the claimed invention. Applicants have also discussed the potential mechanism on page 5, beginning on line 3, through to page 6, lines 1-8.

In view of the foregoing, Applicants submit that claims 1-11, 14-40 and 53-89 satisfy 35 U.S.C. § 122, first paragraph, and as such, are allowable. Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejections Under 35 U.S.C. § 102

Claims 1-11 are rejected under 35 U.S.C. § 102(b) as being anticipated by Holford *et al.* (*Structure*. 1998 Aug 15; 6(8):951-6; PTO 1449 filed 6/15/2001).

Applicants respectfully traverse. However, in order to compact and expedite prosecution, claim 1 has been amended. Holford *et al.*, do not teach or disclose "a first carboxy-terminal portion of a split intein (C-intein), a second amino-terminal portion of a split intein (N-intein), and a target peptide flanked on one end with the carboxy-terminal portion of a split intein (C-intein) and on its other end with the amino-terminal portion of a split intein (N-intein)..." As such, Holford *et al.*, fail to teach each and every claim limitation of the instant invention. These amendments are made solely for purposes of responding to this Office Action and the amendments are made without prejudice or disclaimer. Applicants reserve the right to pursue the subject matter of the original claims in one or more Continuations or Divisional applications.

Further, Applicants maintain that Holford *et al,* does not teach or disclose the use of N-terminal and a C-terminal end of an intein as taught by applicants. Holford *et al.* speculate on how head-to-tail cyclized recombinant peptides and proteins could be made using the taught Expressed Protein Ligation (EPL), where introduction of an N-terminal cysteine and a C-terminal thioester within the same polypeptide chain allows for **intramolecular native chemical** ligation; and that this process has been used to prepare synthetic circular protein domain (see entire document, especially p. 955, penultimate paragraph). A thioesterified protein, **required** for expressed protein ligation, as discussed by Holford, is **only possible** in a **synthetic** milieu and would not be recognized by the skilled artisan as being equivalent to an N- or C-terminal portion of an intein. Holford *et al.*, **requires** the introduction of a cysteine and a thioester. See, for example, page 955, first column, last line of the second paragraph:

> It is also worth noting that introduction of both an N-terminal **cysteine** and a C-terminal **thioester** within the same polypeptide chain allows intramolecular native chemical ligation reactions to be performed, a process which has been used to prepare a synthetic circular protein domain. It is easy to conceive how head-to-tail cyclized recombinant peptides and proteins could be obtained in an analogous manner using EPL. (Citations omitted; Emphasis added).

Holford *et al.*, do not teach an intein as taught by applicants. Rather, Holford *et al.*, introduce an N-terminal cysteine and a C-terminal thioester which allows for an intra molecular ligation. These two **residues** are not inteins, but rather substitutions.

Applicants teach that a first portion and a second portion is an N-terminal portion of a split intein and a carboxy-terminal portion of a split intein wherein each portion alone has no activity and requires the assembly of these portions to form an active enzyme. The number of amino acids that comprise each portion is not important, but the fact that assembly of each portion must form an **active** enzyme. Applicants further teach examples of split inteins. See, for example, page 6, lines 16-19:

> Both the **first portion** of a split intein and the **second portion** of a split intein can be derived from a naturally-occurring split intein such as Ssp DnaE. In other variations, one or both of split intein portions can be derived from non-naturally occurring split inteins such as those derived from RecA, DnaB, Psp Pol-I, and Pfu inteins. (Emphasis added).

Applicants teach that the N- and C-terminal inteins are **proteins that actually associate** to form a complex that initiates and drives the cyclization reaction. (See for example, page 18, lines 5-23 through to page 2, lines 1-12 and figures 1 and 2). Within this complex the cyclization reaction occurs with the **concomitant loss of the N- and C-terminal inteins**. Holford *et al.*, **do not teach** or suggest how to circularize a peptide molecule. Furthermore, Holford *et al.*, do not teach "a nucleic acid molecule encoding a polypeptide comprising a **first portion of a split intein, a second portion of a split intein,**

and a target peptide interposed between the first portion of a split intein and a second portion of a split intein."

Applicants submit that Holford *et al.*, does not anticipate each and every limitation of claims 1-11 and as such claims 1-11 are allowable over Holford *et al.* In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejections Under 35 U.S.C. § 103

Claims 14-40 and 53-89 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Guan et al. (U.S. Patent 5,643,758) in view of Holford *et al.* (*Structure.* 1998 Aug 15; 6(8):951-6; PTO 1449 filed 6/15/2001).

Applicants respectfully traverse.

Applicants have amended the independent claims. Holford *et al.*, do not teach or disclose "a first carboxy-terminal portion of a split intein (C-intein), a second amino-terminal portion of a split intein (N-intein), and a target peptide flanked on one end with the carboxy-terminal portion of a split intein (C-intein) and on its other end with the amino-terminal portion of a split intein (N-intein)..." Further, as discussed previously and reiterated herein, Holford *et al.*, do not teach an intein as taught by applicants. Rather, Holford *et al.*, introduce an N-terminal cysteine and a C-terminal thioester which allows for an intra molecular ligation. These two **residues** are not inteins, but rather substitutions. Holford requires synthetic processing to create a thioesterified protein, and Holford neither teaches nor suggests a polypeptide, as taught by Applicants, that would be amenable to vectors and cells **and** function as N- and C-terminal inteins. Furthermore, the process discussed by Holford *et al.*, necessarily involves synthetic processing to create a thioesterified protein, and Holford *et al.*, neither describes nor suggests a polypeptide, as presently claimed, that would be amenable to the vectors and cells described in Guan *et al.*,

which would **function as N- and C-terminal inteins**. The two teachings are completely incongruent.

Guan *et al.*, do not teach how to make and purify cyclic peptides using inteins. Furthermore, Guan *et al.*, do not teach the "expression of the nucleic acid molecule in a host system produces the **polypeptide** in a form selected from the group consisting of: (a) a polypeptide that **spontaneously** splices **in the host system** to yield a cyclized form of the target peptide, and (b) a **splicing intermediate** of a **cyclized form** of the target peptide." Guan *et al.*, in fact, teaches away from using inteins as the purification method in Guan *et al.*, requires the use of a linking sequence such that use of blood coagulation Factor Xa is required. See Guan *et al.*, column 6, lines 43-64:

> A DNA fragment coding for a predetermined peptide may be employed to link the DNA fragments coding for the binding protein and protein molecule. The predetermined peptide is preferably one **which recognized and cleaved** by a **proteolytic agent** such that it cuts the hybrid polypeptide at or near the protein molecule without interfering with the biological activity of the protein molecule. One such DNA fragment coding for a predetermined polypeptide is described in Nagai et al., Nature, Vol. 309., pp. 810-812 (1984), the disclosure of which is hereby incorporated by reference. This DNA fragment has the oligonucleotide sequence: ATCGAGGGTAGG and codes for the polypeptide Ile-Glu-Gly-Arg. This polypeptide is cleaved at the carboxy side of the arginine residue using blood coagulation factor Xa. As noted above the **linking sequence**, in addition to providing a convenient cut site, may also serve as a polylinker, i.e. by providing **multiple restriction sites** to facilitate fusion of the DNA fragments coding for the target and binding proteins, and/or as a spacing means which separates the target and binding protein which, for example, allows access by the proteolytic agent to cleave the hybrid polypeptide. (Emphasis added).

Guan *et al.*, do not teach or disclose use of inteins for the purification of a peptide that has been generated by the methods of the invention; nor the production of a polypeptide that **spontaneously** splices **in the host system** to yield a cyclized form of the

target peptide, and (b) a **splicing intermediate** of a **cyclized form** of the target peptide. Furthermore, Guan *et al.*, require the use of an **external** agent to cleave the linker molecule.

Applicants submit that, it would not be obvious to one of skill in the art to use Guan *et al.*, in view of Holford et al., to arrive at a method of purifying a cyclic peptide as taught by applicants or the motivation to do so. In fact Guan *et al.*, teach away from use of inteins.

In view of the foregoing, claims 14-40 and 53-89 are allowable under 35 U.S.C. § 103(a) over the cited references. Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Applicants have made every effort to present claims which distinguish over the cited art, and it is believed that all claims are now in condition for allowance. However, Applicants request that the Examiner call the undersigned (direct line 561-671-3666) if anything further is required by the Examiner prior to issuance of a Notice of Allowance for all claims.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks and reconsideration and withdrawal of all rejections. It is respectfully submitted that this application with claims 1-11, 14-40 and 53-89, is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

Although, Applicants believe that no further extensions of time (beyond the two month petition) are required with submission of this paper, Applicants request that this submission also be considered as a petition for any extension of time if necessary. The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for any retroactive extensions of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

Respectfully submitted,

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